rRNA bacterial gene and fungal ITS metagenomics samples

Introduction

The GSAF uses a design based on this tech note from Illumina for reading of the V4/V5 bacterial 16S rRNA gene region, and has extended it to other useful metagenomics primers. This design is a nested PCR in which the first (inner) PCR primes the gene-specific region and adds the Illumina sequencing primer sites, then the second (outer) PCR adds Illumina indexes and flow cell binding/amplification sites. The Illumina sequences are “Nextera” not “TruSeq”.

rRNA gene-specific primer sequences are shown in this teal color.

Illumina platform-specific sequences are shown in this green color.

Dual-barcode sequences are shown in this orange color.

NOTE that all primer pairs are shown here FWD: 5'-3', REV: 3'-5'

Bacterial 16S rRNA V1/V2 primers (version 1):

Inner (first) round:

Hyb8F_rRNA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTGATCMTGGCTCAG-3'
Hyb338R_rRNA: 3'-GTAGGATGCCCTCCTGGACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5'

So the OLIGOS for ORDERING are...

Hyb8F_rRNA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTTGATCMTGGCTCAG-3'

Hyb338R_rRNA: 5'-GTCTCGTGAGGGCTGAGATGTGTATAAGAGACAGTGCTCTCCGAGGATG-3'

Hyb8F is a 3 bp truncation of the classic 27F primer and has been used in many NGS studies such as this one. Hyb338R is a 3 bp truncation of the classic 338R primer. These primers amplify a 343 bp region in E. coli.

GSAF Metadata for this primer set

"fragment_length":343, "linker_primer_sequence":"GTTTGATCMTGGCTCAG", "reverse_primer_sequence":"TGAGGATGCCCTCGG""

Bacterial 16S V4 primers (version 1) - recommended to avoid amplification of Eukaryotic 18S, especially helpful in host/pathogen systems:

Hyb515F_rRNA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTATAAGAGACAGGAGGCTCGGGTGCTCTG-3'
Hyb806R_rRNA: 3'-TAATCTWTGGGVHCATCAGGAGACTACHVGGGTWTCTAAT-5'

So the OLIGOS for ORDERING are...

Hyb8F_rRNA: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTATAAGAGACAGGAGGCTCGGGTGCTCTG-3'

Hyb338R_rRNA: 5'-GTCTCGTGAGGGCTGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT-3'

These are based on, "Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies" by Yong Wang and Pei-Yuan Qian, 2009. These primers amplify a 292 bp region in E. coli.

GSAF Metadata for this primer set
Bacterial 16S rRNA V4/V5 primers (version 1) - these amplify Eukaryotic 18S rRNA!!!:

Inner (first) round:

Hyb515F_rRNA: 5' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTA -3'

Hyb909R_rRNA: 3' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCTCGGGTGCTCTG -5'

These are based on, "Conservative Fragments in Bacterial 16S rRNA Genes and Primer Design for 16S Ribosomal DNA Amplicons in Metagenomic Studies" by Yong Wang and Pei-Yuan Qian, 2009.

In E. coli (O157:H7) these primers amplify a total region of 414 bp from the 16S gene with the final sequencing library being exactly 485 bp.

GSF Metadata for this primer set

Fungal genome ITS primers (version 1) (ITS-1F and ITS-2 as shown under "Fungal Internal Transcribed Spacer (ITS)" on this very helpful primer maps page from Matthew Nelsen) are also available - these should NOT be used for certain dinoflagellate genomes:

HybITS-1F_rRNA, 5' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAGTAA -3'

HybITS2_rRNA, 3' - CGTAGCTACTTCTTGCGTCGAGAGAATATGTGTAGAGGCTCGGGTGCTCTG -5'

In Candida albicans SC5314 these primers create a 256 bp amplicon in ITS1 with the total amplicon with hyb regions being exactly 323 bp. The ITS-1F primer is rooted in the 18S locus while ITS2 is in 5.8S.

ITS primers (version 1) for dinoflagellate Symbiodinium genomes based on the work of Pochon et. al.:

ITS2alg-F, 5' - TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTG -3'

ITS2alg-R, 3' - TTCGTATATTCATTCGCCTCCAGAGAATATGTGTAGAGGCTCGGGTGCTCTG -5'

Barcoding primers, common to all designs:

Outer (second) round primers (examples of two specific indexes are shown, both 5'->3'):

Hyb_F01_i5, AATGATACGGCGACGCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTG

ITACAC GTCTCGCGAGGCGT
This paper is an excellent resource from the SILVA group on Bacteria, Archaea, and Eukaryota. Here is a link directly to their main supplemental table of primer designs and coverage.